Construction of tissue microarrays from core needle biopsy - a systematic literature review

Running title: Tissue Microarrays Using Core Needle Biopsy

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Conflict of Interest Statement: The authors declare that there are no conflicts of interest.

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Word count. (3085)

Abstract

Aims: Core needle biopsy (CNB) in some clinical circumstances may be the only source of material from cancer tissue available for diagnostic purposes. The volume of tissue available in a CNB specimen is low and opportunities for research use can therefore be limited. The Tissue microarray (TMA) principle, if applied to the use of CNB specimens, could facilitate research studies in circumstances where only CNB samples are available. However, various challenges are expected in applying such a technique in CNB which has limited its use in research. We have conducted a systematic review of the literature on this subject.

Methods and Results: A systematic search was carried out using CINAHL, EMBASE, Cochrane library and MEDLINE identifying studies which have primarily developed methods for constructing TMAs from CNB. Eight studies were found to meet the inclusion criteria; six of these employed the vertical rearrangement technique and two used multiple layers of biopsy tissue. Representation of the CNB was significantly influenced by the quantity of tumour cells present on the original biopsy and the degree of heterogeneity of biomarker expression.

Conclusion: Technologies have been developed to enable construction of TMAs from CNB. However, challenges remain to improve amplification and representation.

Keywords: Core needle biopsy, tissue microarrays, biopsy TMA, CNB TMA

Introduction:

In some clinical circumstances surgery may not be the preferred form of initial or primary therapy for breast cancer and a core needle biopsy (CNB) specimen will be the only available material from cancer tissue for diagnostic and research purposes. The volume of tissue and tumour in CNB specimens is far lower than tumour samples obtained from surgically resected specimens which significantly reduces opportunities for tissue based biological research studies. Methods to support biological studies in these clinical circumstances are clearly needed, for example to identify and validate the role of improved predictive biomarkers of therapy efficacy. Non-operative therapy, typically systemic therapy, is used in metastatic setting, as a neoadjuvant therapy to down-stage the disease prior to surgery, and as an alternative therapy to surgery, particularly in patients with co morbidities. For instance, about 40% of older women (>70 years) with early-stage breast cancer receive non-operative therapy, in the form of primary endocrine therapy (1-3), because they refuse or are unfit/frail due to concomitant co-morbidities.

Numerous published studies indicate high levels of concordance in biological characteristics observed between CNB and subsequent surgical excision or excision biopsy (4-8). A meta-analysis carried out by Li *et al,* (2012) indicated high correlation in such key biomarkers of breast cancer biology including oestrogen receptor (ER), progesterone receptor (PR) and human epidermal receptor (HER)2 (6). Similar findings were seen in the estimation of Gleason score in prostate cancer patients (9, 10). Given such evidence for high concordance between CNB and surgical specimens, analysis of detailed biomarker expression in tissue

obtained by CNB would have great research potential. Although CNB provides enough tissue material for initial diagnosis, detailed profiling of the tumour requires a larger volume of tissue, which usually necessitates the use of tissue derived from surgical specimens. Therefore, if CNB were to be used for achieving this purpose, a technique that can maximise the utility of available tissue are required. Tissue microarray (TMA) techniques have been developed, for the purpose of high throughput immunohistochemical profiling of large cohorts of cases using limited amounts of tumour tissue (11). In addition it allows the analysis and evaluation of tissue-based assays in an efficient, cost-effective and uniform manner (12, 13).

The length and thickness of the tissues obtained by CNB makes the conventional TMA method developed by Kononen *et al,* (1998) inappropriate for achieving the same objective using CNB. The small diameter of the biopsy material makes horizontal embedding less effective in amplifying the number of possible sections. Moreover, the biopsy diameter is often eroded after sectioning for initial diagnosis, thus, only a few sections would become obtainable.

The current use of CNB is usually restricted to diagnostic purposes. Therefore, optimising a method for constructing TMAs from CNB presents an opportunity for researchers to profile tumours using a scarce source of tissue. On the other hand, given the potential technical challenges in such construction, it would be necessary to review the literature to identify and critically appraise different methods employed for that purpose.

This study aimed to systematically review the literature for studies which had explored the technology of constructing TMAs from paraffin-embedded CNB and critically analyse their results.

Methods and search strategy

The review of literature was carried out and presented following the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guideline (14). Electronic searches were used to review the literature for related studies. The studies were screened for their titles. The abstracts were then evaluated to determine the studies that would fit the selection criteria, and finally, full articles of the eligible studies were reviewed.

Electronic searches and information source

A comprehensive search was carried out using CINAHL, EMBASE, Cochrane library and MEDLINE. The references of the studies were also screened for any other relevant studies.

Keywords

Key words used for electronic search were identified using Medical Subject Headings (MeSH), including; "tissue microarrays AND core needle biopsy", "construction AND tissue microarrays AND core needle biopsy", "tissue microarrays AND needle biopsy" and "tissue micro arrays AND core needle biopsy".

Inclusion criteria

Primary studies which developed methods for constructing TMAs from paraffin-embedded CNB were included. The search was restricted to work published in English language and between 1998 – January 2015.

Exclusion criteria

Studies utilising a conventional approach to constructing TMA blocks, as described by Kononen *et al* (1998), were excluded.

Type of tissue

Only CNB obtained from human tissues was considered.

Outcome measures

Primary outcome:

The primary objective was to evaluate methods for construction of TMAs from CNB.

Secondary outcomes:

The review also:

- evaluated representativity (homogeneity, antigenicity and morphology) of tissue obtained from TMA blocks based on CNB;
- investigated the number of obtainable sections; and
- investigated the density of cores per block.

Results

The search databases and the number of studies associated with identified keywords are summarised in Table 1. Figure 1 shows a flow chart illustrating different phases of the systematic review. Finally, a summary of the outcome measures is given in Table 2.

Description of included studies

Eight studies were found to meet the inclusion criteria (Table 2). Jhavar *et al* (2005) and Datta *et al* (2005) were the earliest to have published methods for construction of TMAs from CNB (15, 16). Their methods used the most representative area of the biopsy and re-arranged it vertically in the recipient TMA block. Jhavar's method was later modified by McCarthy *et al* (2009), in terms of performing extraction biopsy from the donor block and the number of cores which could be included in the recipient block (17). Later, McCarthy *et al* (2011) utilised a manual arrayer for the purpose of producing high-density TMA blocks (18). Vogel and Bultmann (2010) and Fridman *et al* (2011) applied similar principles used by Jhavar *et al* (2005) with several modifications; this led to an increase in the number of TMA cores (19, 20). Furthermore, Fridman *et al* (2011) utilised all the tissue available from biopies whereas others limited the tissue selection to those enriched for tumour cells. As an alternative to the vertical re-arranging technique, Kishen *et al* (2011) and Komiya *et al* (2013) employed techniques which included embedding multiple layers of horizontal representative segments of the biopsy into the recipient TMA block (21, 22). However, there is a distinct difference between the two techniques, where Kishen *et al* (2011) arranged the multiple layers one over the others, whereas Komiya *et al* (2014) arranged the layers on a single line.

Method for constructing TMAs from CNB

Developed methods for constructing TMAs from CNB consist of two steps; extracting the biopsy from the donor block and integrating the extracted biopsy

within the recipient TMA block (Figure 2). However, for the purpose of ensuring an accurate positioning of biopsy segments within the recipient block and increasing the number of cores included in the TMA block, some techniques included an intermediate step that involved vertical embedding biopsies into an intermediate block.

Jhavar *et al* (2005) started by cutting paraffin blocks to obtain a segment of cubic wax containing the biopsy, which was arranged in steel mould holding the biopsy in a vertical position. McCarthy *et al* (2009) modified the method of Jhavar *et al* (2005) in two aspects; first, by using pre-designed knives for extracting the biopsies of consistent length from the donor block; and second, by utilising a rubber template to design the recipient TMA block. These modifications allowed the construction of a TMA block with an extra capacity for cores and to allow easy extraction of tissue from the donor blocks. A further modification to the method used by Jhavar *et al* (2005) was performed by McCarthy *et al* (2011) and aimed to construct high-density TMA blocks. They used a manual tissue arrayer to punch and transfer biopsies from a pre-constructed intermediate block into the recipient block. The intermediate block was made in accordance with the method of Jhavar *et al* (2005) used originally for constructing the TMA block.

Datta *et al* (2005) used un-fragmented biopsy segments. After extracting the biopsy, from the donor block using a scalpel, it was embedded in a pre-designed template in order to reform wax around the biopsy in a cylindrical shape. Biopsies were then transferred into the TMA blocks which were designed using a manual arrayer. As an alternative to extracting the biopsy from the donor block by scalpel, Vogel and Bultmann (2010) used a skin biopsy punch to obtain tissues enriched with tumour cells from the donor block. Their method included melting wax around

extracted tissue on a hot plate at 65 ^{0}C prior to transferring it to the TMA block. Studies utilising vertical rearrangement techniques were found to integrate tissue cores within the TMA block either by annealing the TMA or melting the TMA blocks and subsequent cooling it.

Fridman *et al*, (2011) utilised a method where case selection was limited by the presence of a minimum of 30% of tumour cells in the biopsy. Their method included melting the original wax block, then staining the tissue cores with Eosin for better visulisation of the extracted tissue segment. Following this, each biopsy was fragmented into equal parts and re-embeded vertically in an intermediatary block. A manual arrayer was then used to transfer cores from the intermediatary block to the TMA block.

Kishen *et al* (2011) applied a multiple-layer approach for making a biopsy TMA block which included embedding two segments of a biopsy (2mm each) on top of the other. The tissue used was confirmed microscopically to be rich in tumour cells prior to making the TMA block.

Komiya *et al* (2013) started with cutting a thick section (30μm) from the donor block, then segments rich in tumour with size of 3mm were dissected from the section and arranged in line on a paraffin sheet of 100-μm thickness. Both tumour segments and paraffin sheet were rolled up into cylindrical reels. The cylindrical reels were then divided into sections and transferred into a pre-designed recipient block.

Representation of tissue obtained from the biopsy TMA block

Representation here refers to three aspects, namely, homogeneity between cores obtained from different biopsies of each patient, preservation of antigenicity and morphology of the original tissue.

Jhavar *et al* (2005) found that antigenicity was fully preserved in tissue obtained from prostate CNB, where their investigation included staining tissue obtained from 123 individuals known to have prostate cancer. Antigenicity was investigated by evaluating three markers used for routine diagnosis, namely, low molecular weight keratin (CAM5.2), high molecular weight keratin (LP34) and prostate specific antigen (PSA). Similar findings were reported by Fridman *et al* (2011) who stained the produced sections with high-molecular weight cytokeratin (HMWCK), PIN-cocktail (p63+p504S), and PSA. Their work demonstrated that morphological and immunohistochemical chractaristics of original tumour CNB were maintained in TMA format.

Datta *et al* (2005) used three cores rich in prostatic carcinoma cells from each individual CNB in order to provide a better estimation of protein expression. Seventeen sets of CNB and the corresponding radical prostatectomy specimens were stained for Ki67 antigen to investigate concordance between the two types of specimen and demonstrated an estimated correlation coefficient (*r)* of 0.4994 (p=0.04).

A tumour detection rate of 66-79% was achieved from TMA blocks constructed from CNB obtained from two series of prostate cancer patients (n=303) using the method described by McCarthy *et al* (2009).

Vogel and Bultmann (2010) reported a high concordance between sections obtained from biopsy TMAs, excision biopsy and mastectomy specimens, in terms of HER2 status in breast cancer. Investigations included using immunohistochemistry (IHC),

fluorescence in situ hybridisation (FISH) and automated brightfield double *in situ* hybridisation (BDISH) techniques. Results of IHC showed minor variations between different tissue types, whereas FISH and BDISH produced identical estimates of ErbB-2 gene amplification.

Komiya *et al* (2013) demonstrated the representativity of CNB in TMAs by showing the prognostic value of Ki67, p53 and bcl-2 in prostate cancer based on the correlation between the results of IHC of these biomarkers and survival data of 58 patients.

Number of cores and obtainable sections

The capacity of a TMA block to include biopsy samples depends on the diameter of cores and distance between cores used. For instance, Jhavar *et al* (2005) who included the integration of biopsy and surrounded wax with the TMA block, produced a smaller number of cores that could be inoculated in the final TMA block. On the other hand, Vogel and Bultmann (2010) managed to include a large number of samples by removing the paraffin surrounding the biopsy segments. In addition, methods such as that from McCarthy *et al* (2011), which used a manual arrayer, were found to create a TMA block with higher capacity. This review indicates that the number of TMA cores ranged from 20 to 187 per TMA block.

Methods for constructing TMA from CNB were found to have standardised CNB length of 3 to 4-mm. The number of obtainable representative sections was influenced directly by the amount of tumour cells present in the original biopsy. The thickness of sections used for IHC staining ranged between 3 and 5-μm (15-21).

Discussion

The length and thickness of donor CNB was found to influence successful tissue extraction from the donor block and the integration of the cores within a recipient TMA block. The conventional method for constructing TMAs developed by Kononen *et al* (1998) has been limited by the need for relatively large amounts of tissue (11). The literature review indicates two approaches for use of CNB in TMA construction, namely, vertical re-arranging and a multiple layer approach utilised to 'amplify' tissue.

An embedded biopsy with multiple fragments with surrounding wax would preserve the original structure of the biopsy. In addition, transferring biopsies with their surrounding wax would be easier and safer to maintain tissue structure than handling the biopsy itself. Moreover, an advantage of this method is to have better integration with the TMA block as wax surrounding the biopsy would easily anneal to the TMA block. Nevertheless, obtaining a biopsy from the donor block with surrounding paraffin would result in a lower number of cores being included in a final recipient block due to the wider area required. However, using a pre-designed tool to extract the biopsy in order to reduce the amount of paraffin surrounding the biopsy might improve the capacity of the TMA block to include more cores. A method for supporting a higher density TMA block includes using the use of a manual or automated arrayer for taking cores from the CNB. The limitation of this practice occurs with non-straight embedded cores or a slightly deviate biopsy from the vertical position; punching biopsies from donor blocks with such defects can result in less tissue being transferred into the recipient block.

Extraction procedures involving the melting of the whole donor block helps achieve a straighter biopsy although the accuracy in re-orientating the original embedded

biopsy might be lost, leading to the possible misplacement of the pre-selected area rich in tumour cells. Instead of melting the whole block, extracting and melting the segment rich in tumour cells would avoid destroying the original material and help re-form and integrate the biopsy within the TMA block.

Biopsies can sometimes contain a small amount of tumour cells (less than 4mm) which is not enough to be embedded vertically on the TMA block. Alternatively, in such circumstances, multiple-layers can be extracted from CNB biopsies and embedded one over the other. For this method TMA cores with the required length are therefore needed to produce a reasonable number of sections. However, stratified cores might contain intra-layer gaps leading to sections without representative tissue. Embedding multiple-layers in parallel or in-line on the surface of the recipient blocks would not help to amplify material from these blocks as the depth of tissue on sections will be similar to the thickness of original biopsies.

Integration of the biopsies with its surrounding wax in the recipient block is a critical step of constructing the CNB TMA, as it is associated directly with biopsy orientation and quality of the section. The techniques reviewed herein are found to apply two methods for integrating biopsies with surrounding wax, namely; i) Softening the recipient block, and/or ii) Melting the whole recipient block and re-solidifying it. Although the softening method clearly helps to even the surface of the recipient block, this method would not ensure that transferred cores into the recipient block are completely annealed with the surrounding wax. One of the advantages of melting the donor block compared to the softening method is having perfectly integrated tissue with the surrounding wax. However, orientation of the

tissues within the recipient block is still an issue, and further work should focus on preserving tissue during integration step.

It is important that sections obtained from biopsy TMAs represent the tumour characteristics of the whole tumour. Issues seen with "the standard method by Kononen *et al.* (1998)" such as intra-tumour heterogeneity might affect the assessment of tumour biology. For instance, the proliferation marker Ki67 expression has shown considerable heterogeneity between different tumour areas (23, 24). Researchers developing TMAs have considered two technical strategies to avoid misrepresentation of the original tumour result due to small TMA cores, namely, ensuring a proper core diameter and the number of cores from each tumour case.

The diameter of tissue taken by CNB usually ranges between 1.2 and 2.5-mm. Moreover, most studies that have constructed biopsy TMA blocks used complete cross sections of the biopsy. An extensively sectioned biopsy for initial tumour diagnosis might have smaller tissue spots. Singh *et al* (2007) suggested that fewer tumour cells available in TMA sections led to reduced representativity of the section and adversely affected the reliability of biopsy TMAs (25). Intra-tumour variations in biomarker expression among different tumour cells could result in reduced concordance between tissues from CNB and surgical specimens in terms of protein expression. For conventional TMAs, sampling strategy proves that three cores or more would properly represent the whole tumour tissue and reduce error associated with tumour heterogeneity (26-28). Datta *et al* (2005) investigated the optimal number of cores from each individual biopsy for better representation of the whole tumour in patients with prostate cancer, demonstrating that tumour biology could be accurately estimated using three core biopsies from each patient

(16). However, the number of cores per block depends on the degree of biomarker expression heterogeneity. It is also important to mention that there are situations where CNB should not be subjected to harvesting for TMA. Small invasive tumour tissue within the core or extensively sectioned CNB for initial tumour diagnosis with the potential of tumour depletion can be considered as a contraindication to TMA. This is to ensure preservation of diagnostic core material for subsequent review whenever needed particularly in relatively recent cases. Another contraindication to CNB TMA is the lack of representation of the index invasive tumour such as CNBs from invasive carcinoma that contain pure ductal carcinoma in situ (DCIS),DCIS associated with microinvasion, or tiny invasive tumour tissue that are unlikely to be presented on TMA cores.

Conclusion

Constructing TMAs from CNB requires modification of the conventional methodology. Further developments are required to improve amplification of tissue from the biopsy. Owing to the issue of tumour heterogeneity as identified in the studies reviewed, further work should also focus on enhancing the representativity of tissues obtained from biopsy TMAs.

Acknowledgment

The review was conducted as part of ALBANGHALI MA's PhD funded by Al-Baha University, Ministry of Education, Saudi Arabia.

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Titles and legends to Tables

Table 1: Summary of a number of studies identified for different keywords and databases.

Database	Keywords	Number of articles found
CINAHL	Tissue microarrays AND core needle biopsy	0
	Construction AND Tissue microarrays AND core needle biopsy	0
	Tissue microarrays AND needle biopsy	0
	Tissue micro array AND core needle biopsy	0
Cochrane library	Tissue microarrays AND core needle biopsy	2
	Construction AND Tissue microarrays AND core needle biopsy	0
	Tissue microarrays AND needle biopsy	2
	Tissue micro array AND core needle biopsy	0
MEDLINE	Tissue microarrays AND core needle biopsy	20
	Construction AND Tissue microarrays AND core needle biopsy	5
	Tissue microarrays AND needle biopsy	58
	Tissue micro array AND core needle biopsy	3
EMBASE	Tissue microarrays AND core needle biopsy	3
	Construction AND Tissue microarrays AND core needle biopsy	0
	Tissue microarrays AND needle biopsy	45
	Tissue micro array AND core needle biopsy	0

Table 2: Summary of characteristics of included studies**.**

Number	Authors	Tumour site	Number of cores/ TMA block	Core Measures	No. of obtainable sections (thickness)	Tissue loss	Representativity	Concordance
1	Datta *et al*, (2005)	Prostate	60	1.5-mm Ø, 2-mm	50-150 (5-µm)	-	**Marker:** Ki-67, assessed using IHC.	Ki-67 was over estimated on core needle biopsy compared to radical prostatectomy; the coefficient correlation between two sources of tissue was (r=0.4994, P=0.04).
2	Jhavar *et al*, (2005)	Prostate	45	4-mm	90-100 (4-µm)	9%	**Marker:** Low molecular weight keratin (CAM5.2), high molecular weight keratin (LP34), prostate specific marker (PSA) and H&E, assessed using IHC. **Antigenicity:** 100% preserved.	-
3	McCarthy *et al*, (2009)	Prostate	Maximum 72	4-mm	-	-	**Marker:** p63/AMACR, Ki-67 and Hif1-α . **Gene amplification:** ERG gene status was investigated using FISH. **Cancer detection rate:** 66-79%	No difference in marker expression was seen when compared with an old method (Jhavar *et al,* 2005).
4	Vogel and Bultmann, (2010)	Breast	Maximum 187	1.4-mm Ø, 4-mm	600 (3-µm)	1 to 2% of tissue rolling or folding	**Marker:** HER2, assessed using IHC. **Gene amplification:** ErbB-2, assessed using FISH and BDISH.	- Concordance was investigated between sections obtained from core needle biopsy and mastectomy specimen. - IHC results showed minor variations. - FISH and BDISH showed identical estimate of ErbB-2 gene amplification.
5	McCarthy *et al,* (2011)	Prostate	Maximum 104	1.5-mm Ø, 4-mm	-	-	-	-
6	Kishen *et al*, (2011)	Breast	18	0.6-mm Ø, 4-mm	More than 100	-	-	-
7	Fridman *et al*, (2011)	Prostate	-	0.5 to 0.6-mm Ø, 3 to 4-mm	80-100 (4-µm)	No evident of tissue loss	**Marker:** High-molecular weight cytokeratin (HMWCK), PIN-cocktail (p63+p504S), and Prostate specific marker (PSA), assessed using IHC.	-
8	Komiya *et al*, (2013)	Prostate	20	3-mm Ø, 3-mm	>100 (4-µm)	-	**Marker:** Ki-67, p53 and bcl-2 were assessed using IHC.	-

(IHC) Immunohistochemistry; (FISH) Fluorescence in situ hybridization; (BDISH) Bright field in situ hybridisation.

Titles and legends to figures

Figure 1 Summary of the search process and results of literature review of construction of tissue microarrays from core needle biopsy.

Figure 2 Approaches for constructing tissue microarrays from core needle biopsy.